Selective Targeting and Thermal Destruction of Live Cells Using Antibody Functionalised Gold Nanoparticles

A thesis presented for the degree of

Doctor of Philosophy

by

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Certificate of Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Glossary of Acronyms

2^{o}abFITC	Secondary IgG antibody conjugated with FITC
$AgNO_3$	Silver nitrate
AlOOH	Aluminium oxide hydroxide
Au	Gold
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
Cat. No.	Catalogue Number
CD8	Cluster of differentiation 8
CD11b, CD11b-Mac1	Rat rat anti-mouse monoclonal antibody
CHO-K1	Chinese hamster ovary cells
CTAB	Hexadecyltrimethylammonium bromide, also known as cetyltrimethylammonium bromide
DMEM-F12	Dulbecco's modified Eagle's medium/Ham's nutrient mixture F-12
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTC	Dithiocarbamate
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EGFR	Epidermal growth factor receptor
EPR	Enhanced permeability and retention
FBS	Fetal bovine serum, alpha-immediate heat
FITC	Fluorescein isothiocyanate
GLUT1	Glucose transporter type I
$HAuCl_4$	Chloroauric acid
HCl	Hydrochloric acid

HEPES	Hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HER2	Human epidermal growth factor receptor 2
HNO_3	Nitric acid
HOC	Human oral squamous cell carcinoma
IgG	Immunoglobulin G
KBH_4	Potassium borohydride
KOH	Potassium hydroxide
MPS	Mononuclear phagocytic system
NIR	Near infrared
NPs	Nanoparticles
PBS	Phosphate buffered saline (No Ca^{2+} and Mg^{2+})
PCR	Polymerase chain reaction
PEG	Poly-ethyleneglycol
pI	Isoelectric point
PV	Parasitophorous vacuole
RAW264.7	Murine macrophage cell
RES	Reticuloendothelial system
RPMI1640	Roswell Park Memorial Institute media-1640
SEM	Sacanning electron microscope
SKBR3	Human breast carcinoma cell
T. gondii	*Toxoplasma gondii*
TEM	Transmission electron microscope
U937	Human leukemic monocyte lymphoma cell line

Abstract

Precious metal nanoparticles have attracted considerable interest on account of their actual or potential applications in chemical, biological or medical analyses, and for their applications in various new types of optical devices or systems. These particles can be engineered to absorb light at a particular wavelength and they can also be chemically functionalised to bind to target cells. Active targeting of the gold particles to the site of a disease can be achieved, in principle, by attaching a suitable antibody to the surface of the gold. Localised heating arises when the affected tissue is irradiated with a laser tuned to the plasmon resonance of the nanoparticle because some of the incident laser light is converted to heat in the particle, which then flows out of the nanoparticle into the target cell. This principle is currently being explored overseas as the basis of a novel form of medical treatment for cancer.

In this thesis, I extend this concept to develop a method for selectively killing different cellular targets. I report how gold nanoparticles, either spherical or rod-shaped, were functionalised with specific antibodies so that they would selectively attach to particular target cells: murine macrophage cells and tachyzoites of the protozoan parasite *Toxoplasma gondii*. Following this, the cells were exposed to defined wavelengths and low intensities of continuous laser irradiation from a HeNe laser or a solid state diode laser. Cell viability was determined using nucleic stain dye. Exposure of target cells to specific bioconjugated gold nanoparticles resulted in the highest number of cell death compared with other treatments. In addition, another useful result, independent of the actual process of photothermal therapy, is described in this thesis. This involves the attachment of gold nanoparticle-antibody conjugates to *Toxoplasma gondii* tachyzoites, which clearly reduced their infection of host cells. Therefore, the research described provides both for an exciting and novel possibility for *in vivo* killing of any type of target cells using photothermal therapy and for a means to decrease host cell invasion by an intracellular parasite in the body.